Remarks

I. Support for Amendments

Claim 1 was amended to more clearly define the invention and to correct a typographical error. Support for this amendment is found throughout the specification, for example at page 8, lines 9-16, page 8, line 29 to page 9, line 7, and page 22, lines 1-5. Accordingly, no new matter has been added by these amendments and entry thereof is respectfully requested. Applicants also submit herewith the Declaration of Michael R. Hamblin, Ph.D., pursuant to 37 CFR § 1.132. This Declaration is provided in support of Applicants' arguments traversing the Examiner's rejections.

II. The Objection to claim 1 may properly be withdrawn

The Examiner has objected to claim 1 because of the typographical error in line 3. Applicants have corrected this typographical error to recite "from the". Accordingly, Applicants respectfully request that this objection be withdrawn.

III. The Rejection of claims 1, 5, 7-11, and 20 under 35 U.S.C. § 112, first paragraph may properly be withdrawn

The Examiner has rejected claims 1, 5, 7-11, and 20 under 35 U.S.C. § 112, first paragraph, as he asserts that the specification does not describe the subject matter of these claims in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Specifically, the Examiner asserts that the terms "the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissues or cells" and "the degree of the metachromatic shift of the dye from a library" lack sufficient antecedent basis in

the specification. Applicants respectfully traverse this rejection for the reasons discussed below.

The Examiner's attention is respectfully directed to the Declaration of Michael R. Hamblin, Ph.D., (hereinafter, "Declaration of Dr. Hamblin"), which is being filed herewith. Dr. Hamblin received his Ph.D. in Synthetic Organic Chemistry from Trent Polytechnic, U.K. and has spent more than 27 years involved in the fields of enzyme chemistry, synthetic organic chemistry, and photodynamic therapy. He is currently an Assistant Professor of Dermatology at Harvard Medical School. The Declaration of Dr. Hamblin addresses the outstanding Office Action and presents for the record Dr. Hamblin's considered opinion of what the person of ordinary skill, at the time of the invention of the subject application, would have understood from the specification. The Declaration of Dr. Hamblin notes that one of ordinary skill in the art would understand that support for the terms "the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissues or cells" and "the degree of the metachromatic shift of the dye from a library" may be found, for example, on page 8, lines 9-16 and page 22, lines 1-5.

The Examiner has also asserted that the disclosure at page 8 does not suggest comparing the degrees of the metachromatic shifts of the reflected light spectrum of a dye measured and recorded using a stained tissue or stained cells and a library of previously obtained spectra of similarly stained tissues or cells. The Examiner further asserts that the disclosure at page 22 does not refer to measuring and recording the degree of the metachromatic shift in the reflected light spectra of either a stained tissue or stained cells and a library of previously obtained spectra of similarly stained tissue or similarly stained cells. Applicants respectfully disagree with these assertions. As the Declaration of Dr. Hamblin notes, one of skill in the art would have understood that these phrases are supported by the cited passages of the specification. Furthermore, as stated by Dr. Hamblin in his Declaration, additional support for measuring and comparing the degrees of the metachromatic shifts from the reflected light spectrum of stained tissue or cells with a library of similarly stained tissue or cells is found throughout the specification, for example, at page 8, line 29 to page 9, line 7:

> The (reflectance) spectroscopic analysis of lesions that stain with toluidine blue or with other biological stains or dyes, or with a combination of such stains or dyes...allow for a differential diagnosis of the underlying disease, or disease state of the stained lesion. Cells displaying various stages of metaplasia stain differentially...which is then correlated to the spectrum with a high degree of specificity. This is accomplished by comparing the reflectance spectrum of the stained tissue or lesion with a 'library' or composite of spectrums from lesions that have been similarly stained and subsequently diagnosed by conventional or classical histochemical methods.

Accordingly, as noted by Dr. Hamblin, a person of ordinary skill in the art, upon reading the specification, particularly the passages cited above, would understand that the inventors had possession of an invention directed to a method of diagnosing the degree of metaplasia in biological tissue or cells by obtaining a reflected light spectrum of the stained tissue or cells and comparing the degree of the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue. Accordingly, Applicants respectfully request that this objection be withdrawn.

IV. The Rejection of claims 1, 5, 7-11, and 20 under 35 U.S.C. § 112, second paragraph may properly be withdrawn

The Examiner has rejected claims 1, 5, 7-11, and 20 under 35 U.S.C. § 112, second paragraph, as he asserts that these claims are indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner asserts that the phrase, "comparing the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with the degree of the metachromatic shift of the dye from a library" is vague. The Examiner further asserts that it is not clear what aspect or characteristic of the spectrum is to be compared with the library of the previously obtained spectra, as he asserts that the terms "the metachromatic shift" and "the degree of the metachromatic shift" appear not to be defined in the specification. Applicants respectfully traverse this rejection for the reasons discussed below.

With respect, Applicants point out that the question whether claim terms are indefinite under 35 U.S.C. § 112 must be answered from the perspective of one of ordinary skill, who has read and understood Applicants' specification. As established by Dr. Hamblin in his Declaration, the terms "the metachromatic shift" and "the degree of the metachromatic shift" are sufficiently defined in the specification at, for example, pages 21, line 29 to page 22, line 5, and one skilled in the art would have been familiar with the phenomenon of metachromasia where, for example, a dye stains certain cell components a different color than the original color of the dye. According to Dr. Hamblin, it is also known to those of skill in the art that this metachromasia is quantitated by measuring the extent of the metachromatic shift by comparing the intensity of the light in a desired light spectrum between, for example, two or more specific wavelengths. As explained in the Declaration of Dr. Hamblin, since one of ordinary skill in the art would know how to determine the degree of the metachromatic shift (as described above), one of ordinary skill in the art would also know how to compare these degrees in metachromatic shifts. The metachromatic shift is a change in wavelength measured in nanometers while the intensity is typically measured in absorbance units. Therefore, the sample tissue or cells would be illuminated with light of a known spectrum (i.e., known intensity at every wavelength), and the spectrum of the remitted light would be measured to determine how much light was absorbed at every wavelength. The measured wavelength and intensity of the stained tissue or cells could then be compared to measurements from a library of previously obtained spectra of similarly stained tissue or cells that were illuminated by the same piece of equipment.

Therefore, as explained by Dr. Hamblin, one of skill in the art would have been familiar with metachromatic shifts and how to compare the spectra from a sample tissue or cell with a library of previously obtained tissues or cells. Accordingly, as noted by Dr. Hamblin, the terms "the metachromatic shift" and "the degree of the metachromatic shift" are sufficiently defined so that the skilled artisan is able to determine the metes and bounds of the subject matter that Applicants regard as their invention. Therefore, Applicants respectfully request that this rejection be withdrawn.

The Examiner also asserts that the phrase "with a library of previously obtained spectra of similarly stained tissue or cells" is vague and indefinite because it cannot be determined from which similarly stained tissue or cells said library of previously obtained spectra is to be obtained prior to steps (a)-(d), and from what source said similarly stained tissue and cells are to be derived. Applicants respectfully traverse this rejection for the reasons discussed below.

As stated in the Declaration of Dr. Hamblin, one of ordinary skill in the art, upon reviewing the specification (specifically page 8, line 29 to page 9, line 7, and page 21, line 29 to page 22, line 5), would understand that the library of previously obtained spectra would include those cells and/or tissues that are the subject of the particular analysis and would be obtained from individuals that may exhibit the particular cellular abnormality. The Declaration of Dr. Hamblin provides numerous examples of different skin cancer types from which the cells and tissues in the library may be obtained. Accordingly, as noted by Dr. Hamblin, based on the disclosure in the specification, along with the knowledge of one of skill in the art, the phrase "with a library of previously obtained spectra of similarly stained tissue or cells" is clear to the skilled artisan who would be reasonably apprised of the metes and bounds of the claims. Nevertheless, in a sincere attempt to advance prosecution, Applicants have amended claim 1 to recite the fact that the library of similarly stained tissue or cells have been previously diagnosed by conventional methods and exhibit the particular disease state being diagnosed. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The Examiner also contends that claims 1, 5, and 7-11 are vague and indefinite because, he asserts, the phrase "correlating the reflected light spectrum with a disease state" is vague and indefinite. The Examiner also asserts that it cannot be ascertained how correlating the reflected light spectrum with a disease state leads to a diagnosis of dysplasia, pre-cancer, or cancer in a living organism, since it cannot be determined how the spectrum of the reflected light and disease state are related. The Examiner further asserts that the specification does not appear to guide one to compare the extent of the metachromatic shift in the test sample spectra with that obtained from a library of previously obtained spectra of similarly stained tissue or cells, which have been

previously diagnosed by conventional techniques. Applicants respectfully traverse this rejection for the reasons discussed below.

As explained in the Declaration of Dr. Hamblin, one of skill in the art would understand that the basic mechanism of a metachromatic shift involves the dye's interaction with individual cell components that are differentially stained. This concept is explained throughout the specification, including, for example, at page 3, line 20 to page 4, line 25, and page 4, line 27 to page 5, line 2. The Declaration of Dr. Hamblin also notes that the specification at page 8, lines 14-20, further describes how dysplastic, pre-cancerous, and cancerous lesions are differentially stained by the metachromatic dyes. In addition, the specification at, for example, page 8, line 29 to page 9, line 7 and page 22, lines 1-5, clearly explains how one can compare the extent of the metachromatic shift in the test sample spectra with that obtained from the library of previously obtained spectra of similarly stained tissues or cells. The specification also describes how the samples upon which the library is based have been previously diagnosed by conventional techniques, such that a correlation as to the disease state of the test sample may be made by such comparison of the metachromatic shift of the test sample with that of the reference sample.

Therefore, as noted by Dr. Hamblin, the phrase "correlating the reflected light spectrum with a disease state" is sufficiently defined as that phrase would be understood by the ordinarily skilled artisan. Notwithstanding this, in a sincere attempt to advance prosecution, Applicants have amended claim 1 to clarify that the *degree of the metachromatic shift of the dye* from the reflected light spectrum is correlated with a disease state. Accordingly, Applicants respectfully request that this rejection be withdrawn.

The Examiner also asserts that claims 1, 5, and 7-11 are indefinite because of the term "similarly stained tissue or cells." The Examiner opines that it cannot be ascertained how similarly the different tissue or cells may be stained. With respect, and as explained by Dr. Hamblin in his Declaration, one of ordinary skill in the art would be aware that, in order to compare staining results from separately stained tissue/cell samples, the same standardized protocol would have to be used to stain the two samples. Notwithstanding

this knowledge of the skilled artisan, in order to advance prosecution, Applicants have amended claim 1 to recite that the tissue or cells in the library are stained according to the same protocol as the stained tissue or cells to be diagnosed. Accordingly, withdrawal of this rejection is respectfully requested.

V. The Rejection of claims 1, 5, 7-11, and 20 under 35 U.S.C. § 102(e) may properly be withdrawn

The Examiner has rejected claims 1, 5, 7-11, and 20 under 35 U.S.C. § 102 as being anticipated by Cabib et al. (U.S. Patent No. 5,784,162), as evidenced by Vaezy et al. (*Journal of Microscopy*, 163: 85-94, 1991), and Marchesini et al. (*Photochemistry and Photobiology*, 55: 515-522, 1992) for the reasons set forth in the previous Office Actions. In these Office Actions, the Examiner has asserted that Cabib's description of spectral imaging methods for *in situ* medical diagnosis and treatment comprising preparing a sample to be imaged, viewing a sample through an optical device optically connected to a spectrometer, collecting and measuring incident light using a detector and collecting and interpreting data using a mathematical algorithm, form a proper basis to reject the claims as anticipated. In the Office Action of November 26, 2001, the Examiner also asserted that 1) "numerous examples of *in situ* analyses of cells and/or tissues to either classify and/or diagnose cellular abnormalities in said cells and/or tissues are provided" in Cabib et al., and Examples 1, 6, 7 and 8 are pointed to with particularity; 2) Cabib et al. "discloses that a metachromatic dye, such as Azure-B, which is a thiazine dye, can be used to practice the prior art methods (see Example 2, column 43, line 10);" 3) "the sample of tissue or cells to be analyzed [in Cabib et al.] is prepared by staining with either Romanowsky-Giemsa stain, haematoxylin-eosin stain, or May-Grunwald-Giemsa stain (see claim 59), each of which are compositions comprising thiazine dyes;" 4) Cabib et al. teaches "that a spectral component may 'correlate well with what is called the purple Romanowsky-Giemsa complex,'" and 5) "an objective of the prior art invention is

to distinguish cancer from healthy or otherwise diseased tissue or cells (column 6, lines 27-33)." Applicants traverse this rejection for the reasons discussed below.

Applicants will first briefly describe the claimed invention and further explain why, as established by Dr. Hamblin, Cabib et al. does not teach or suggest, either expressly or inherently, Applicants' invention, and will respond to the present Action with respect to the Examiner's contentions previously urged in rebuttal. In light of the discussion below, it is believed that the above rejections of record have been overcome and that all pending claims are in condition for allowance. Action toward this end is respectfully solicited.

A. The Invention

As understood from the specification and as noted in the Declaration of Dr. Hamblin, the present invention relates to methods for diagnosing dysplasia, pre-cancer or cancer *in situ* in biological tissue or cells of a living organism that include utilization of spectroscopic methods to analyze the metachromatic properties of various dyes in abnormal (e.g., dysplastic, pre-cancerous and cancerous) and normal cells. The inventors of the present invention have surprisingly discovered that the extent of the metachromatic shift observed in a dye from stained tissue or cells can be used to differentiate, for example, the aforementioned abnormal cells and/or tissues from normal cells and/or tissues. According to one embodiment of the methods described in the application, the metachromatic shift of a dye observed in the reflected light spectrum from a dye-stained test sample is compared to the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells wherein the diagnosis of the disease state of the reference cells or tissue upon which the previously obtained spectra are based was confirmed by conventional histopathology methods, such as histochemical methods. Thus, quicker, more precise diagnoses may be made according to the present method compared to existing methods.

B. Applicant's position

1. Cabib et al. fail to teach the claimed invention

As stated by Dr. Hamblin in his Declaration, the disclosure of Cabib et al. does not expressly or inherently teach or suggest quantifying the extent of metachromatic shift of a metachromatic dye in a stained test sample and making a correlation with a dysplastic, pre-cancer or cancer disease state using this information as recited in the pending claims. Dr. Hamblin opines that one of ordinary skill in the art who read Cabib et al. would have been given no hint to even try quantifying the extent of metachromatic shift of a metachromatic dye in a stained test sample and making a correlation with a dysplastic, pre-cancer or cancer disease state using this information as recited in the pending claims. Dr. Hamblin notes that Cabib et al. is an extremely broad and general disclosure which describes numerous spectral imaging methods for biological research and medical diagnostics. However, as pointed out by Dr. Hamblin, nowhere in Cabib et al. is there any mention or suggestion of metachromasia or the utilization of the metachromatic shift using the methods of the present invention.

For instance, as explained in the Declaration of Dr. Hamblin, the Cabib et al. reference primarily relates to detecting spatial organization and quantifying cellular and tissue natural constituents and does not teach or suggest methods of diagnosing dysplasia, pre-cancer or cancer by quantifying, for example, the metachromatic shift of a metachromatic dye. Dr. Hamblin explains that Cabib et al. are relying on the inherent spectra of biological components in making a determination of cancer, not use of dyes as described in applicants' specification. For instance, as pointed out by Dr. Hamblin, at column 1, lines 17-21, Cabib et al. states that [t]he methods of the present invention can be used to detect spatial organization (i.e., distribution) and to quantify cellular and tissue natural constituents, structures, organelles and administered components such as tagging probes (e.g., fluorescent probes) and drugs...." It is further stated in column 6, lines 36-39, of Cabib et al. that "[t]he method further enables the identification and spatial mapping of proteins, sacharides [sic], AND+ [sic] and NADH, collagen, elastin and flavin, and various additional metabolic mediators within cells and/or tissues."

Additionally, it is stated in column 6, lines 27-33, that "[a]nother objective of the present invention is to map in a quantitative way white light, ultraviolet or laser-induced emission spectra from biological components (e.g., oxygenated and deoxygenated hemoglobin in retinal blood vessels and or melanin pigmentation level in the retina) and, to distinguish cancer from healthy, or otherwise diseased tissue or cells." Based on these statements, as well as the rest of the disclosure in the Cabib et al. reference, Dr. Hamblin concludes that Cabib et al. are relying on the spectra of the biological components in making a determination of cancer.

Furthermore, in the Office Action of December 18, 2002, the Examiner asserted that Cabib et al. (a) teaches that "morphometric spectral image analysis enables evaluation of subtle cytological and histological features to yield useful ultrastructural and medical information for diagnostic and prognostic evaluation," (b) discloses that "[s]ince various malignancies are also characterized by unique developmental features, the SpectraCube™ system and the methods of the present invention can be adopted to monitor these characterizing features and thus to assist in for example early diagnosis (e.g., existence and stage) of such malignancies;" and (c) discloses an example (Example 8) in which the SpectraCube™ system and the methods of the claimed invention were used to differentiate a cancerous cell from a normal cell. With respect, Applicants dispute each of these unsupported assertions by the Examiner.

As stated by Dr. Hamblin in his Declaration, the description of the SpectraCube™ system, as with the entirety of the Cabib et al. disclosure, is too broad and general as to provide the person of ordinary skill with any suggestion or description of the methods of the present invention. Therefore, one of ordinary skill in the art would understand that The SpectraCube™ system described by Cabib et al. does not describe or suggest the utilization of metachromasia in making a diagnosis of dysplasia, pre-cancer or cancer in situ for the reasons discussed below.

The Examiner's attention is particularly and respectfully directed to the statement of Dr. Hamblin that Cabib et al. describes the use of dyes as *contrast agents to visualize structures* (e.g., to look at biological components and/or their spectrum) and *does not describe or suggest the use of metachromasia for any diagnostic purpose*. For instance,

the "morphometric spectral image analysis" relates to applying spectral imaging to improve the quantitative measurement of the size, shape and textural features of cells as demonstrated in Example 2. In Example 2, the dye is used as a contrast agent to visualize cellular structures. When a dye is used as a contrast agent, the intensity of the light absorbed by the stained sample is measured in order to differentiate the various cellular components that are stained at varying intensities by the same dye. On the other hand, the analysis of the metachromatic shift of a dye according to the claimed invention, focuses on the color whereby certain cell components and/or cell types are actually stained a different color than the original color of the dye. Dr. Hamblin further explains that use of the SpectraCube™ system involves the quantitation of euchromatin and heterochromatin and the morphological analysis of cytoplasmic components.

Furthermore, Dr. Hamblin explains that Example 8 of the Cabib et al. patent describes the staining of a cervical smear with haematoxylin-eosin for aiding in the diagnostic pathology as analyzed by a transmission miscroscopy RGB image. Since haematoxylin stains acidic structures and eosin stains basic structures, *the stains in this example are simply being used as contrast agents* in order to quantitate various cellular structures to aid in the diagnosis. Accordingly, as noted by Dr. Hamblin, one of ordinary skill in the art, at the time of the filing of U.S. Patent Application No. 09/306,662, would understand that the Cabib et al. description does not describe or suggest utilizing the metachromatic properties of a dye to diagnose cancer as recited in the pending claims.

In his Declaration, Dr. Hambin also opines that one skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teaching of Cabib et al. For instance, Dr. Hamblin explains that Cabib et al. describes the use of a stain composition that includes two dyes - eosin Y and azure B and notes that there is no suggestion that a metachromatic shift is being used to diagnose dysplasia, pre-cancer or cancer, especially since a non-metachromatic dye (eosin Y) is being used and because Cabib et al. teach a method based on contrast rather than metachromasia.

In fact, the Declaration of Dr. Hamblin notes that *it would not even be possible to utilize a metachromatic shift in the method of Cabib et al.* because the combination of the

two dyes would result in the presence of multiple colors, thereby interfering with an examination of the change in color of the single metachromatic dye. Accordingly, as noted by Dr. Hamblin in his Declaration, since the method of Cabib et al. measures two colors from two different dyes rather than examining differential color staining from a single metachromatic dye, there is no description or suggestion in Cabib et al.of the present invention.

2. Response to current Action's assertions

a. Cabib et al. relate to detecting spatial organization and quantifying cellular and tissue natural constituents, and any method of diagnosing cancer discussed in Cabib et al. involves use of such information, not information relating to quantitating the metachromatic shift of a metachromatic dye

With all due respect, it appears to Applicants that the Examiner, in the outstanding Action, appears to continue to misunderstand the import of Applicants' arguments made in the Supplementary Response mailed August 23, 2002 and the Response and Amendment mailed May 19, 2003. The outstanding Action asserts that the teachings of Cabib et al. include methods of diagnosing cancer, and asserts that Applicants argue that Cabib et al. relate to detecting spatial organization or distribution and quantifying the cellular or tissue constituents, not diagnosing cancer. However, on page 4 of Applicants' Supplementary Response, lines 7-10, Applicants stated "Cabib et al. relate to detecting spatial organization and quantifying cellular and tissue natural constituents and any method of diagnosing cancer discussed in Cabib et al. involves use of such information, not information relating to quantifying the metachromatic shift of a metachromatic dye." *The importance of the emphasis on the methods of Cabib et al. being used to detect and quantify cellular and tissue natural constituents was that Cabib et al. are relying on the inherent spectra of biological components in making a determination of cancer, not use of dyes as claimed by Applicants.* Therefore, as noted in the Declaration of Dr. Hamblin, although the methods of Cabib et al. may or may not be

useful to diagnose cancer, Cabib et al. does not describe or suggest a method of diagnosing dysplasia, pre-cancer or cancer in situ in biological tissues or cells by comparing the metachromatic shift of stained tissues or cells with a library of similarly stained tissues or cells that have been previously diagnosed by conventional methods.

b. One skilled in the art would not recognize that metachromasia could be used in a diagnosis of dysplasia, pre-cancer or cancer from the teachings of Cabib et al.

The Action also states that "it had long been appreciated in the art that thiazine dyes, such as methylene blue and toluidine blue, display metachromasia; i.e., they stain certain cell components a different color than the original color of the dye." Therefore, the Examiner concludes that "'metachromasia' is nor more than a fanciful way of describing the change in the absorption or transmission spectrum of a dye that occurs after staining two different types of cells or tissues." The Examiner further asserts that, "[t]he 'metachromatic shift of the dye' to which the claims refer, thus, is interpreted to denote the change that is observed in the transmission spectrum of a dye after staining a particular tissue or cell relative to that which was previously observed after staining another tissue or cell, or relative to a composite of transmission spectra observed after staining a library of tissues or cells." The Examiner concludes that, "[t]he change in the absorption or transmission spectra of a dye, or the metachromatic shift is an inherent property of the dye." Therefore, the Examiner asserts that there is "no manipulative difference between the steps practiced in performing the prior art's disclosed methods for diagnosing cancer." The Examiner also concludes that "in practicing the method of the prior art, the artisan necessarily determined the metachromatic shift of the dye that was used to stain the tissue or cells." Applicants respectfully but strongly disagree with the Examiner's facile, incorrect and unsupported characterization of "metachromatic shift" for the reasons discussed below.

According to the Examiner's incorrect definition of "metachromasia," the "metachromatic shift of the dye" is argued to denote the change that is observed in the

transmission spectrum of a dye after staining a particular tissue or cell. By contrast, as noted by Dr. Hamblin, the methods of the present invention will be appreciated to measure the *reflectance* spectrum rather than the *transmission* spectrum. In addition, as noted in the Declaration of Dr. Hamblin, the Examiner fails to define or otherwise support exactly what this asserted "change" in the transmission spectrum actually is or how it is to be measured. By contrast, the present invention quantifies and compares the observed metachromatic shifts. Furthermore, the Examiner incorrectly asserts that the metachromatic shift is an inherent property of the dye. In actuality, as expressed by Dr. Hamblin in his Declaration, the metachromatic shift is not an inherent property of the dye, but is a property of the interaction between the dye and the tissue or cell that is being stained.

Furthermore, as explained in the Declaration of Dr. Hamblin, Cabib et al. never uses the term "metachromatic shift" or even suggests the utilization of the metachromatic interaction between the dyes and the stained samples. Furthermore, Cabib et al. provides no description of a metachromatic shift and provides no suggestion of quantifying the degree of such a shift. As explained by Dr. Hamblin, one of ordinary skill in the art would understand that the technology disclosed in Cabib et al. does have the ability to measure the metachromatic shifts of dyes; however, the fact that these shifts were not measured suggests to one of ordinary skill that Cabib et al. did not appreciate the potential of using metachromasia in making diagnoses. The additional fact that the Cabib et al. reference is extremely broad and includes a number of applications of morphometric spectral imaging, without including any reference to the use of metachromatic shifts, further suggests that Cabib et al. did not recognize the potential use of these shifts in diagnoses. In fact, that recognition comes only from improper hindsight, based on the Applicants' specification disclosure.

In addition, as described in the Declaration of Dr. Hamblin, it would not even be possible to utilize a metachromatic shift in the method of Cabib et al. because Cabib et al. describes the use of a stain composition that includes two dyes - eosin Y and azure B. The combination of these two dyes would result in the presence of multiple colors, thereby interfering with an examination of the change in color of the single

metachromatic dye. Since the method of Cabib et al. measures two colors from two different dyes rather than examining differential color staining from a single metachromatic dye, it is not true that, in practicing the methods of Cabib et al., the artisan necessarily determined the metachromatic shift of the dye that was used to stain the tissues or cells, as asserted by the Examiner.

c. Passages from Cabib et al. cited in the Office Action, as well as the teachings of Cabib et al. as a whole, support the conclusion that the methods of Cabib et al. rely on the inherent spectra of biological components and the use of dyes as contrast agents in diagnosing cancer

The Examiner further asserts that the prior art's methods for diagnosing cancer comprise measuring and recording the spectral shifts, so that the practitioner need not rely upon his or her visual faculties to distinguish between often too subtle differences in those shifts. Therefore, the Examiner concludes that the dyes of the present invention and of the prior art are still used as contrast agents, but the differences in the reflective spectra of the stained tissues or cells is measured using an instrument, rather than by eye. As discussed in the Declaration of Dr. Hamblin, there is a difference in using a dye as a contrast agent versus analyzing the metachromatic shift of a dye and its interaction with a cell or tissue. Dr. Hamblin explains that when a dye is used as a contrast agent, the intensity of the light absorbed by the stained sample is measured in order to differentiate the various cellular components that are stained at varying intensities by the same dye. On the other hand, Dr. Hamblin explains that the analysis of the metachromatic shift of a dye focuses on the color whereby certain cell components and/or cell types are actually stained a different color than the original color of the dye.

The Examiner also disagrees with the Applicants' definition of the term "in situ." Applicants respectfully disagree with the Examiner's characterization of this term for the reasons discussed in the previous responses and incorporated herein. Nevertheless, regardless of the definition given to this term, Cabib et al. do not describe the methods of

the present invention for all the reasons described herein as well as in the Declaration of Dr. Hamblin.

d. Cabib et al. teach it is preferable not to use dyes in the methods described therein

The Examiner continues to disagree with Applicants' position that Cabib et al. teach that it is preferable not to use dyes in the methods described therein. In response to the Examiner's assertions, Applicants respectfully disagree based on the arguments submitted in our previous responses and incorporated herein. Nevertheless, assuming *arguendo*, that Cabib et al. does not teach that it is preferable not to use dyes in the methods described therein, these dyes are still used as contrast agents as opposed to being used for any metachromatic properties. As described the Declaration of Dr. Hamblin, there is a difference in using a dye as a contrast agent versus analyzing the metachromatic shift of a dye and its interaction with a cell or tissue. Furthermore, as discussed above, Dr. Hamblin explains that it would not even be possible to utilize a metachromatic shift in the method of Cabib et al. because Cabib et al. never describe the use of a single metachromatic dye. Rather, Cabib et al. describe the use of a stain composition that includes two dyes - eosin Y and azure B. As explained in the Declaration of Dr. Hamblin, the combination of these two dyes would result in the presence of multiple colors, thereby interfering with an examination of the change in color of the single metachromatic dye as performed by the methods of the present invention.

Therefore, as noted by Dr. Hamblin in his Declaration, Cabib et al. do not teach or suggest the methods of the present invention because it fails to teach the use of a metachromatic shift in diagnosing dysplasia, pre-cancer, or cancer in situ in biological tissue or cells as described above. Moreover, as explained by Dr. Hamblin in his Declaration, one of ordinary skill in the art would not recognize that metachromasia could be used in a diagnosis of these conditions based on the disclosure in Cabib et al.; in fact, it would not have been possible to utilize the metachromatic properties of the dyes using the methods of Cabib et al. Accordingly, as noted by Dr. Hamblin in his Declaration, Cabib et al., as would be understood by the person of ordinary skill in the art

at the time of the filing of the application, do not describe or suggest, either expressly or inherently, the method as recited in the claims of the pending application for the reasons discussed above. Hence, Applicants respectfully request that this rejection be withdrawn.

VI. The Rejection of claims 1, 5, 7-11, and 20 under 35 U.S.C. § 103(a) may properly be withdrawn

The Examiner has also rejected claims 1, 5, 7-11, and 20 under 35 U.S.C. § 103(a) as being obvious over U.S. Patent No. 5,784,162 to Cabib et al., in view of Tuite et al. (*Journal of Photochem. and Photobiol. B: Biol.*, 21: 103-124, 1993), as evidenced by Vaezy et al. (*Journal of Microscopy*, 163: 85-94, 1991), and Marchesini et al. (*Photochemistry and Photobiology*, 55: 515-522, 1992). The Examiner relies on Cabib et al. for describing methods of using a combination of dyes comprising methylene blue. However, the Examiner admits that Cabib et al. do not teach the use of toluidine blue O. The Examiner also relies on Tuite et al. for describing that toluidine blue O can selectively stain tumor cells and that it is generally non-toxic to normal cells. The Examiner concludes that it would be obvious to have used toluidine blue O in the methods of Cabib et al. because both methylene blue and toluidine blue O had been characterized as non-toxic and are known to selectively stain cancer cells. The Examiner further asserts that one of ordinary skill in the art would have been motivated to use toluidine blue in the methods of Cabib et al. to confirm the results of analyses in which methylene blue had been used. Applicants respectfully traverse this rejection for the reasons discussed below.

Applicants have already stated the deficiencies of the Cabib et al. reference above. As stated in the Declaration of Dr. Hamblin, Tuite et al. discuss photochemical interactions of methylene blue and analogues with DNA and other biological substrates. Specifically, methylene blue, Azure B, Azure A, Azure C, thionine and toluidine blue O are discussed. Dr. Hamblin explains that because all of these dyes described in Tuite et al. are non-toxic and can selectively stain cancer cells, one skilled in the art would not

have been motivated to select toluidine blue O to confirm the results of his or her analysis as asserted by the Examiner.

Furthermore, as explained in the Declaration of Dr. Hamblin, a metachromatic shift can only be detected when a single metachromatic dye is used and cannot be detected with a combination of dyes. However, Cabib et al. never describes or suggests the use of methylene blue by itself. The only mention of a methylene blue related dye is Cabib et al.'s description of the Romanowsky-Giemsa and Haematoxylin-Eosin staining techniques, which utilize a *combination* of Azure-B and Eosin Y. Therefore, since Cabib et al. never describes or suggests the use of methylene blue by itself, no metachromatic shift could have been observed using the methods of Cabib et al. and it would not have been obvious to use this dye by itself in order to observe such a metachromatic shift.

Furthermore, as noted by Dr. Hamblin, even if one of skill in the art were to select toluidine blue O for use in the methods of Cabib et al., it would simply be used as a contrast agent. For the reasons discussed above, and contrary to the Examiner's assertions, such use as a contrast agent would be different from the methods of the present invention, which compare the degree of the metachromatic shift of the dye from the reflected light spectrum of the stained tissue or cells with the degree of the metachromatic shift of the dye from a library of previously obtained spectra of similarly stained tissue or cells.

Furthermore, as noted by Dr. Hamblin, the Vaezy et al. (*Journal of Microscopy*, 163: 85-94, 1991), and Marchesini et al. (*Photochemistry and Photobiology*, 55: 515-522, 1992) references do not supply the deficiencies of the primary reference to describe or suggest the methods of the present invention. Therefore, as noted by Dr. Hamblin in his Declaration, one of ordinary skill in the art would understand that there is no description or suggestion in any of these combined references of the method recited in the present invention for the reasons discussed above. Accordingly, Applicants respectfully request that this rejection be withdrawn.

VII. CONCLUSION

In view of the foregoing remarks, Applicants believe that the application is in condition for allowance. However, if the Examiner disagrees, he is encouraged to call the undersigned at the number listed below in order to expedite the prosecution of this application.

Respectfully submitted,

Henry N. Wixon.
Reg. No. 32,073

Date: 8 OCTOBER 2004

Wilmer Cutler Pickering Hale and Dorr LLP
1455 Pennsylvania Ave., NW
Washington, D.C. 20004
(202) 942-8459